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Department of Cardiac Function in LCAD Deficient Mice After a Single Bout of Endurance Exercise

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ASSESSMENT OF CARDIAC FUNCTION IN LCAD DEFICIENT MICE AFTER A SINGLE BOUT OF ENDURANCE EXERCISE

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Abstract

Fatty acids are the primary fuel source for cardiac tissue in both humans and animals. These become especially important during times of starvation and long bouts of exercise. Fatty acids are broken down into smaller, useable acyl-CoA subunits through a process called beta-oxidation. The first step in this process must be catalyzed by one of four acyl-CoA dehydrogenase enzymes depending on the length of the fatty acid to be metabolized. The enzyme that catalyzes the breakdown of long-chain fatty acids, long-chain acyl-CoA dehydrogenase (LCAD), was examined. The objective of this study was to determine how a single bout of endurance exercise impacts cardiac function in LCAD deficient mice as well as to assess whether recovery from exercise is adversely affected. Mice were separated into four groups for testing: LCAD, non-exercised; LCAD, exercised; control, non-exercised; and control, exercised. Mice were exercised by forced running at a speed of 31 m/min with increasing grade (2%) each 20 min. Twenty-four hours post-exercise, the mice were anesthetized with sodium pentobarbital (40 ml/kg body weight,) and ejection fraction, fractional shortening, and cardiac output values were determined in both groups. It was determined that exercise had no effect on cardiac function or recovery in either the LCAD or control group. There was, however, a significant difference between the LCAD and control group for fractional shortening (p<0.05) and cardiac output (p<0.05). It was concluded that a single bout of endurance exercise had no significant effect on the cardiac function of LCAD deficient mice during recovery, although the disease did negatively affect cardiac function when compared to the control group.

Introduction

In order to perform its numerous tasks, the body must constantly produce useable energy from chemical compounds such as carbohydrates, proteins, and fats. Fats, specifically fatty acids, are stored as triglycerides in both adipose tissue and skeletal muscle until they are needed for energy production. The energy stored in these compounds is released through a process known as beta-oxidation (Schulz, 1991). Because the body depends on numerous sources of fuel, including fats, to supply it with energy, beta-oxidation plays a key role in the regulation and maintenance of metabolic homeostasis in the body. Fatty acid degradation in the mitochondria is an essential process for energy production during periods of fasting and other metabolic stresses.

Fatty acids broken down through beta-oxidation produce adenosine triphosphate (ATP) that is used by the body to maintain physiologic function, particularly in cardiac tissue and skeletal muscle. ATP produced via oxidation of fatty acids is primarily depleted by these tissues during times of prolonged exercise and also plays a major role in supplying energy while the body is at rest (Coyle, Jeukendrup, Wagenmakers and Saris 1997). The ATP produced from fatty acids via beta-oxidation, the Krebs cycle, and electron transport may also fuel the body in times of fasting in order to help regulate the body's blood glucose levels since glucose, a molecule that can be oxidized to produce ATP, is no longer being supplied by the diet (Schulz, 1991).

Within the last thirty years, people have begun to be diagnosed with deficiencies of ACDs and other enzymes that affect fatty acid metabolism (Ventura, Ruiter, Ijlst, Tacares de Almeida, and Wanders, 1998). Although cases of fatty acid oxidation disorders (FODs) are rare when compared with other metabolic diseases, the effects of these disorders can be very severe and are often fatal in younger patients. FODs are now known to be among the most common of genetically inherited metabolic disorders and frequently present the individual with life-threatening conditions. While more information is being uncovered about FODs, there is still much that is unknown about them due to their rare occurrence in humans.

The presence of an LCAD deficiency in mice produces symptoms that are most closely associated with those seen in humans with a VLCAD deficiency (Kurtz et al. 1998a). Characteristics of LCAD deficiency in mice may include sudden death, fasting and cold intolerance, hypoketotic-hypoglycemia, and an increase in the fatty content of the liver and heart. Furthermore, LCAD deficiency may be a cause of skeletal myopathy in matured mice as well. Exercise has been hypothesized to play a part in the causation or stimulation of all the symptoms mentioned above (Strauss et al., 1995; Mathur et al., 1999). LCAD deficient mice also experience excessive gestational loss of both LCAD -/- (40%) and LCAD +/- (50%) pups. While much is known about LCAD deficiency in humans and mice, there are relatively few published studies.

While use of mice with LCAD deficiency is common, a human case of the deficiency has yet to be found. This may be due to an increased risk of gestational loss as seen in the mouse model (Schuler & Wood et al. 2002). One of the most common FAO disorders found in humans is that associated with very-

long chain fatty acids (VLCAD). Symptoms associated with human VLCAD deficiency during infancy include nonketotic hypoglycemic coma, cardiac hypertrophy, endocardial fibroelastosis, hepatomelagy, Reye-like syndrome, sudden infant death syndrome (SIDS), and lipid storage myopathy (Hale & Bennett, 1992). While VLCAD deficiencies are the most prominent and easily diagnosed type seen in humans, onset of the disorder during adolescence or early adulthood is still very difficult to detect. Mouse models of FOD have introduced the opportunity to investigate the physiological effects of these deficiencies in humans and may potentially lead to earlier and easier detection. LCAD deficient mice have become a valuable model in the study of triglyceride deposition in both the human and animal heart.

When the oxidation of long-chain fatty acids is impaired, both cardiac and muscle tissues can be severely affected (Olgilvie et al., 1994). There are five studies suggesting that exercise may amplify VLCAD deficiency in humans (Olgilvie et al., 1994; Strausberg et al., 1997; Smelt et al., 1998; Minetti et al., 1998; Merinero et al., 1999). Importantly, the patients in the above studies represented a variety of ages and all complained of abnormal muscle pain and weakness during exercise. Further, evidence from previous experiments indicates that VLCAD deficiency in mice may cause a delay in the recovery of skeletal muscle from exercise. It is unclear whether the delay is associated with a reduced ability to resynthesize ATP and phosphocreatine (energy sources), or the reduced ability to resynthesize muscle glycogen (carbohydrate) stores. It is also unknown whether or not the delayed recovery is associated with either rhabdomyolysis or prolonged fatigue. On the whole, little is known about how exercise affects skeletal muscle and cardiac function in animals with LCAD deficiency.

Another area of interest is whether FOD disorders specifically have an effect on the ejection fraction and fractional shortening capabilities of the heart. The ejection fraction measures the percentage of volume of blood that is ejected with each ventricular contraction. Ejection fraction is calculated using a ratio that compares the volumes of the left ventricle during both systole and diastole (LVdiastole-LVsystole/LVdiastole).

Fractional shortening is another measurement that can be used to assess left ventricular performance. Like the ejection fraction, fractional shortening measures the efficiency of the left ventricle. Instead of using volumes, fractional shortening is based on the change in the diameter of the left ventricle during systole and diastole (LVdiastole-LVsystole/LVdiastole). This method focuses less on volume change and more on cardiac tissue function during both systole and diastole. A heart that is not functioning properly, therefore, would be expected to produce a lower fractional shortening percentage than a normally functioning heart. In the mouse model, the average value for fractional shortening is approximately 57% with values below 40% being indicative of cardiac malfunction or improper left ventricle performance (Gardin et al. 1995). When extrapolated,

the results produced by the mouse model provide a positive correlation with the average known values of the human model.

It was anticipated that the values obtained for ejection fraction, fractional shortening, and cardiac output of LCAD deficient mice would be lower than those seen in wild type controls both during rest and after periods of endurance exercise due to their inability to properly use long-chain fatty acids to provide energy to the heart during these times.

Objective

The objective of this research was to determine if recovery from endurance exercise is delayed in LCAD deficient mice and if cardiac function is adversely affected. The effects of LCAD deficiency and acute exercise on cardiac function were assessed by examining specific cardiac dimensions, the ejection fraction, fractional shortening, and cardiac output.

Materials and Methods

Procedures used in this study were based on the protocol reported by Johnson and Riggs (2002). Cardiac function was assessed via echocardiography in normal (129 SvJ.C57BL6) mice (n=10) and mice (129 SvC57BL6 knockout) homozygous for an LCAD deficiency (n=10). All procedures were identical for each group of enzyme deficient mice (pre- and post-exercise) as well as each control group (pre- and post-exercise). Mice were divided into four groups: 1) non-deficient, non-exercised; 2) enzyme deficient, non-exercised; 3) non-deficient, exercised; 4) enzyme deficient, exercised. Mice were exercised by forced running at 31 m/min with increasing grade (2%) each 20 min. Exercise was terminated upon exhaustion. Twenty-four hours post-exercise the mice were anesthetized and cardiac function was determined via echocardiographic methods. In vivo testing of the contractile properties of the heart was conducted using echocardiography after anesthetizing the mouse using sodium pentobarbital (40 mg/kg body weight) administered interperitoneally. Data were collected before and after exercise in LCAD and control mice and the results were compared both within groups and between groups. The echocardiographic methods used to evaluate cardiac function provided results consistent to those previously reported by Rottman et al. (2003). Stroke volume was determined by tracing the outline of the left ventricle during systole and diastole. Measurements were taken for both short- and long-axis views. Heart rate was determined by placing needle electrodes in the right and left shoulder of the mouse as well as a ground wire in the leg. The highest registered reading was used in the computation of cardiac output. Cardiac output was determined by multiplying the value obtained for stroke volume by the value obtained for heart rate (CO = SV X HR).

Statistical Analysis

Data were analyzed utilizing analysis of variance (ANOVA) procedures in a 2 x 2 factorial design. The factors were enzyme deficiency (LCAD deficient, Control) and exercise (Exercised, Non-exercised). All comparisons were made at the $p < 0.05$ level of significance.

Results

Complete data were collected for n = 10 mice in both the LCAD and control groups for all variables (EF, FS, SV, HR, CO).

Table 1:

Figure 1: A significant difference was present between the control and LCAD groups both pre- (p = 0.001) and post-exercise (p = 0.013).

Echocardiographic Measurements for LCAD Deficient Mice Pre-Exercise						
Category	Name	Units	Mean	SD	Minimum	Maximum
Ejection Fraction	EF		0.864	0.098	0.750	1.000
Fractional Shortening	FS%		61	1.79	58.1	63.6
Stroke Volume	SV	mL	0.05	0.00483	0.04	0.05
Heart Rate	HR	bpm	370.4	22.85	340	410
Cardiac Output	CO	mL/min	17.32	1.146	15.08	18.90

Echocardiographic Measurements for LCAD Deficient Mice Post-Exercise						
Category	Name	Units	Mean	SD	Minimum	Maximum
Ejection Fraction	EF		0.855	0.104	0.750	1.000
Fractional Shortening	FS%		60.9	2.91	57.1	65.6
Stroke Volume	SV	mL	0.05	0.00483	0.04	0.05
Heart Rate	HR	bpm	369.3	27.06	334	415
Cardiac Output	CO	mL/min	17.24	0.860	16.00	18.35

Echocardiographic Measurements for Control Mice Pre-Exercise						
Category	Name	Units	Mean	SD	Minimum	Maximum
Ejection Fraction	EF		0.870	0.148	0.750	1.000
Fractional Shortening	FS%		65.8	2.21	62.2	69.0
Stroke Volume	SV	mL	0.05	0.00483	0.05	0.06
Heart Rate	HR	bpm	361.0	29.13	315	408
Cardiac Output	CO	mL/min	19.04	1.267	17.25	21.36

Echocardiographic Measurements for Control Mice Post-Exercise						
Category	Name	Units	Mean	SD	Minimum	Maximum
Ejection Fraction	EF		0.888	0.099	0.750	1.000
Fractional Shortening	FS%		64.3	2.52	61.2	68.6
Stroke Volume	SV	mL	0.05	0.00421	0.05	0.06
Heart Rate	HR	bpm	369.3	27.06	310	405
Cardiac Output	CO	mL/min	18.95	0.889	17.50	20.25

Ejection Fraction

Ejection fraction values were determined before and after exercise for both experimental groups. The average value of ejection fraction for the LCAD group before exercise was found to be 0.864 ± 0.098 and the average EF after exercise in the same population was 0.855 ± 0.104. Average values of ejection fraction were also obtained for the control group before and after exercise. These values were found to be 0.870 ± 0.148 and 0.888 ± 0.099, respectively.

There was no significant difference between the values obtained for either group as a result of exercise. There was also no significant difference between the LCAD and control groups.

Fractional Shortening

Values for fractional shortening were obtained before and after exercise for both experimental groups. Averages were determined for all groups and are included in Table 1. The average value of FS for the LCAD group before exercise was 61± 1.79 and the average value after exercise was 60.9 ± 2.91. Data were also compiled and averaged for the control group which produced results of 65.8 ± 2.21 (%) and 64.3 ± 2.52 (%) before and after exercise respectively.

There was no significant difference in fractional shortening as a result of exercise. There was, however, a significant difference present between the average values obtained for the LCAD and control groups (p<0.05). A graphical interpretation of the results can be seen in Figure 1 below.

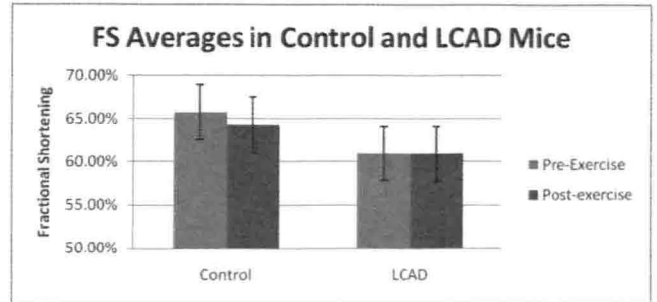## Cardiac Output

Cardiac output values were determined for both LCAD and control groups before and after exercise. The average values for cardiac output for the LCAD group were found to be 17.32 ± 1.146 (ml/min) before exercise and 17.24 ±0.860 (ml/min) after exercise. The control group produced averages of 19.04 ± 1.267 and 18.95 ± 0.889 for the pre- and post-exercise groups, respectively. While no significant difference was found as a result of exercise, there was a significant difference between the LCAD and control groups (p<0.05). Figure 2 shows the mean values of cardiac output of both the LCAD and control mice before and after exercise.
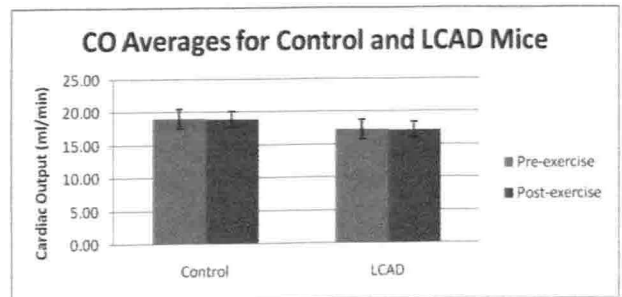
Discussion

Figure 2: A significant difference was present between the control and LCAD groups both pre- (p = 0.005) and post-exercise (p = 0.001).

Variation of data was seen within individuals in both groups and across all variables. Discrepancy in the data produced for each individual can largely be attributed to the natural variation in homeostatic conditions commonly seen in both humans and animals. It is also possible that the condition of the mouse (stress level/activity level) at the time of testing had an impact on cardiac function. However, it is likely that any such effect was minimal. In order to produce a data set more indicative of the test group as a whole, averages were calculated and used for statistical analysis.

The values for ejection fraction for LCAD deficient mice were expected to be below normal during rest and after periods of endurance exercise when compared to the control group. Statistical analysis of the data showed that there was no significant difference in the values obtained within either group

as a result of exercise. It was also determined that there was no significant difference between the LCAD mice when compared to the control group. Ejection fraction aims to measure the volume change seen in the left ventricle during each contraction of the heart. As previously stated, this is done by comparing the volume of the left ventricle during diastole to the volume of the left ventricle during systole. The data provide relevant information on the cardiac function of LCAD mice. It was concluded that a single bout of endurance exercise has no effect on ejection fraction in LCAD deficient mice. It can be deduced that the inability of these mice to use long-chain fatty acids during endurance exercise, a time when fatty acids are the primary fuel source, poses no detrimental effects to cardiac function during recovery.

Data were also collected for fractional shortening in both LCAD and control groups. The means for both experimental groups before and after exercise compared favorably to the expected values presented in studies by both Gardin et al. (1995) and Rottman et al. (2003). Because there was no significant difference between the values obtained for each experimental group as a result of exercise, it can be concluded that LCAD enzyme deficiency has no effect on cardiac function during recovery from exercise in LCAD deficient mice. There was a significant difference, however, in the fractional shortening values of the LCAD group when compared to the control group both before and after exercise. This finding is of particular interest, especially because there was no significant difference in the ejection fraction values obtained from the two groups. The disparity present in the data could possibly point to some type of dysfunction in the cardiovascular system of LCAD deficient mice. A more in-depth examination of fractional shortening in the LCAD mouse population would be necessary to develop further conclusions regarding the reason for the decreased fractional shortening values.

Analysis of the cardiac output values collected from LCAD deficient mice before and after exercise showed that endurance exercise had no significant effect on cardiac output during recovery. This indicates that the inability of LCAD mice to effectively metabolize fatty acids for use in cardiac muscle tissue during endurance exercise had no effect on overall cardiac function after recovery. However, statistical analyses did reveal that cardiac output values obtained for the LCAD group were significantly lower than those of the control group. It is possible that this discrepancy can be explained by the fact that, in general, control mice are heavier than LCAD mice. Because this is true, control mice generally have bigger hearts and would, therefore, be expected to have greater overall cardiac output. Since heart size was not taken into account when calculating cardiac output, a one-way ANOVA was run analyzing the weights of both experimental groups; control mice were indeed significantly larger than LCAD mice ($p < 0.05$). It can be reasonably assumed that, because the control mice are larger, they have larger hearts. This would adequately explain the difference seen in cardiac output measurements between the LCAD and control mouse experimental groups. It cannot be conclud-

ed, however, whether this was the true cause for discrepancy as heart weight was not taken for the two experimental groups.

The data collected for both the LCAD and control groups were similar to those of Rottman et al. (2003). This not only supports previous research on cardiac function in mice, it also further promotes the efficacy of echocardiography as an effective assessment technique for measuring cardiac output in mice. The determination of cardiac output via echocardiographic methods is less intrusive than many of the other practices currently employed in the evaluation of cardiac function in mice.

This research will contribute to the base of research supporting echocardiography, although more is still needed in order for it to become a common tool for analysis of cardiac function in mice. While answers to specific questions regarding cardiac function in LCAD deficient mice have been addressed through this research, it has also raised some additional questions. Research should address whether the cardiac output would be affected if the mice were put through a training regimen rather than a single session of endurance exercise. Research should also examine whether the age of an LCAD deficient mouse plays a role in cardiac function and whether cardiac function of LCAD deficient vs. normal mice would differ if readings were taken directly after exercise. Further, role of heart size in identified differences in CO data between LCAD and control mice could be investigated, along with determination of other physical or physiological reasons that LCAD mice appear to have decreased cardiac output when compared to control mice.

Conclusion

After extensively testing the contractile properties of the mouse heart via echocardiography, it was concluded that LCAD enzyme deficiency has no serious detrimental effects on cardiac function during recovery from exercise in LCAD deficient mice. It was also determined, however, that LCAD enzyme deficiency negatively affects cardiac function in LCAD deficient mice when compared to wild type control mice. LCAD deficient mice consistently displayed lower averages for both fractional shortening and cardiac output than control mice, which is indicative of decreased overall cardiac function.

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Mentor Comments:

In his mentor letter, Professor Charles Riggs points out that Evan Lord's work has implications for understanding how fatty oxidation disorders affect cardiac function.

The work completed by Evan Lord is an extension of work done by previous students with my guidance. He received an Honors College Undergraduate Research grant in the Fall 2008 to begin work on his project. He completed the project in the Spring 2009.

His work concentrated on the effects of fatty acid oxidation disorders and exercise on cardiac function using transthoracic echocardiography. While the technique has been used to evaluate the effects of a variety of conditions on the structure of the heart it has been used sparingly to evaluate cardiac function. Since one of the consequences of fatty acid oxidation disorders is frequently a decline in cardiac function, Mr. Lord decided to determine if echocardiography could be used to determine cardiac function in these mice and to determine if the disorder and the combination of the disorder and exercise would have an effect on function of the heart.

It is clear that fatty oxidation disorders can have significant consequences for people of all ages. Little research has been done examining the effects of exercise on mice with these disorders so his work will make valuable contributions to knowledge about the disease and also about the capabilities of affected animals to exercise.